InnoMol Bioimaging Workshop

Adenovirus-based anti-tumor approaches: a contribution of imaging

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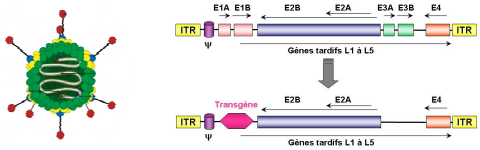
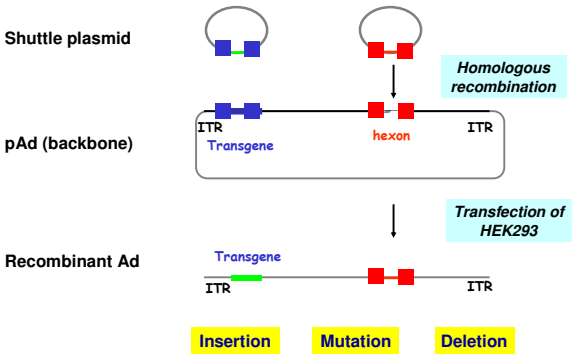
Adenoviral vectors (serotype 5, ΔE1ΔE3)



- Non-integrative
- Transduction of both post-mitotic and dividing cells
- Large cloning capacity (up to 7.5 Kb for ΔE1ΔE3)
- Production at high titer (10^{12} pfu/ml)
- Non-associated with serious human pathologies

Benihoud et al. Curr Opin Biotechnol 1999

Modification of Ad genome by genetic engineering

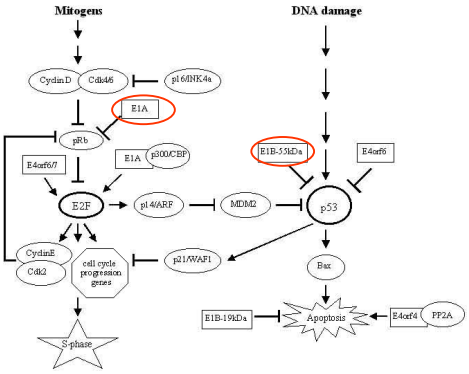


Part 1

Combined therapy of colon carcinomas with an oncolytic adenovirus and valproic acid:

Contribution of confocal microscopy to our understanding of molecular mechanisms

Ad interactions with proteins involved in the control of the cell cycle



Virotherapy based on conditionally replicative Ad (CRAd or oncolytic Ad)

Type I CRAd

	E1A	*E1B19K*	*E1B55K*	*E3*
Onyx-015 (dl1520)	+	+	-	+
AdΔE1B55	+	+	-	-
Δ24 (del923-946)	CR2 mutation	+	+	+
dl922-947	CR2 mutation	+	+	+

Type II CRAd

	Promoter	*E1A*	*Promoter*	*E1B*	*E3*
CV787	Probasin	+	PSA	+	+
CV890	AFP	+		+	+
OBP-301 (Telomelysin)	hTERT	+		+	+

Bressy et al. Biochem. Pharmacol. 2014

Characterization of CRAd replication in preclinical steps

- **Tumor cells and « normal » cells**
- **Human tumors xenografted on nude mice**
- **Other models: Syrian hamster, cotton rat**

Replication

Selectivity

Efficacy

Toxicities

Interaction with the immune system

Limits of oncolytic adenoviruses

- **Insufficient dissemination** in solid tumors (interstitial hydrostatic pressure, complexity of extracellular matrix)
- **Incomplete selectivity** of virus replication into tumor cells
- **Immune components** (neutralizing antibodies, TLR)
- **Non-specific uptake leading to toxicity** due to binding / transduction of non-targeted tissues (liver, blood cells, …)
- **Difficulty to transduce tumor cells** due to low or lack of expression of Ad receptors

Future directions

- **Arming CRAds** with a transgene displaying anti-tumor activity (apoptosis, immunity, killing)
- **Increasing CRAd tropism** for tumor cells (targeting)
- **Decreasing interactions** with blood components (detargeting)
- **Combining CRAds with other therapeutics** (radiotherapy, immunotherapy, chemotherapy)

Combination of CRAds with targeted chemotherapeutic drugs

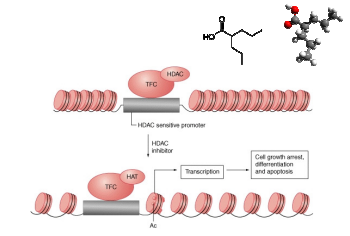
CRAd	Drug	Tumor	Viral replication	Synergy/ additivity	Mechanisms in addition to oncolysis
mTOR inhibitors					
Adtcf-E1AE1B	RAD001	Colon	Unmodified	n.i.	Angiogenesis inhibition
Δ24-FibRGD	RAD001	Glia	Unmodified	Synergy	Autophagy
OBP-405	Rapamycin	Glia	Unmodified	Synergy	Autophagy
Adcyc3-E1A (ΔE1B)	Rapamycin	Breast, lung	Increased	Synergy	Autophagy
dl922-947	Rapamycin	Glia	Reduced	n.a.	Autophagy inhibition
Inhibitors of other kinases					
Δ24-FibRGD	Cetuximab (+5-FU + radiotherapy)	Lung	n.i.	n.i.	n.i.
AdS100A2-E1	Cetuximab	Lung, skin	n.i.	Additivity	n.i.
dl922-947	Bevacizumab	Thyroid	Increased	Additivity	Angiogenesis inhibition/ drop of interstitial pressure
dl922-947	AZD1152	Thyroid	Increased	Additivity	Polyploidy and caspase-3 activation
Onyx-015	CI-1040	Colon	Reduced	n.i.	Cell cycle arrest
Inhibitors of histone deacetylases					
Telomelysin	VPA, FK228	Lung	Increased	Synergy	Increased cell entry
Onyx-015	TSA	Esophagus	Increased	Synergy	Increased cell entry
CN702	VPA	Prostate, colon	Decreased	Antagonism	Cell cycle arrest
Δ24-FibRGD	VPA	Glia	Unmodified	n.a.	n.a.
dl922-947	VPA	Colon	Unmodified	n.i.	Induction of polyploidy

n.a., not applicable; n.i., not investigated.

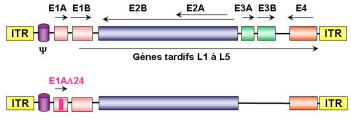
Bressy et al. Biochem. Pharmacol. 2014

Combination of a CRAd and an HDACi

- **HDACi** = Valproic acid (**VPA**)
  - → Apoptosis
  - → Differenciation
  - → Anti-angiogenic



- **CRAd = AdE1AΔ24**



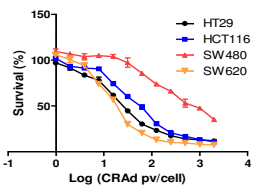
Sensitivity of colon carcinoma cell lines to VPA or AdE1AΔ24

- **VPA**

MTT assay (day 2)

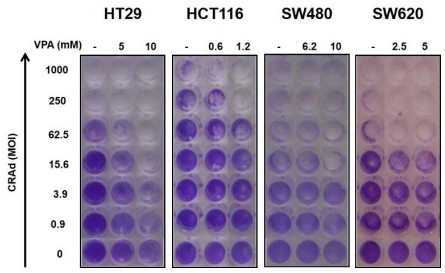
Cell lines	IC50 (mM)	IC25 (mM)
HT29	10	5
HCT116	1.2	0.6
SW480	10	6.2
SW620	5	2.5

- **CRAd = AdE1AΔ24**



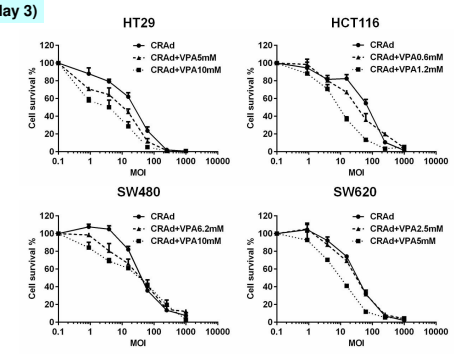
Reduction of colon carcinoma cell line growth after combined treatment with a CRAd and VPA

Crystal violet assay (day 3)



Reduction of colon carcinoma cell line growth after combined treatment with a CRAd and VPA

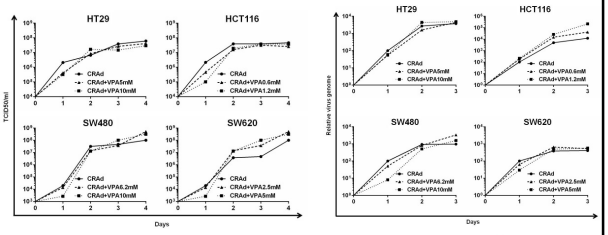
MTT assay (day 3)



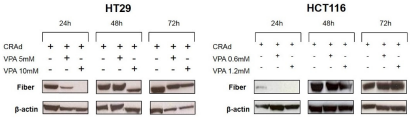
Viral production after CRAd-VPA cotreatment

Virus (TCID50)

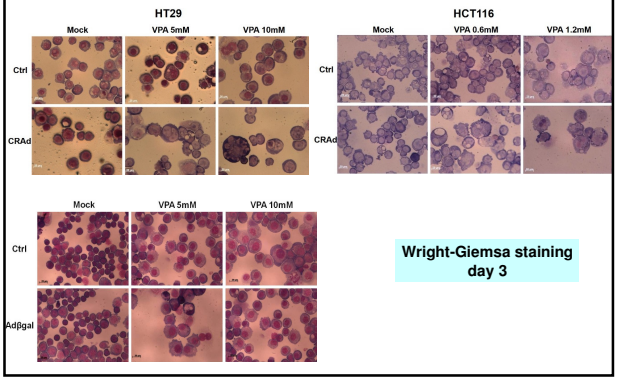
Viral genome (hexon QPCR)



Late protein expression (fiber)

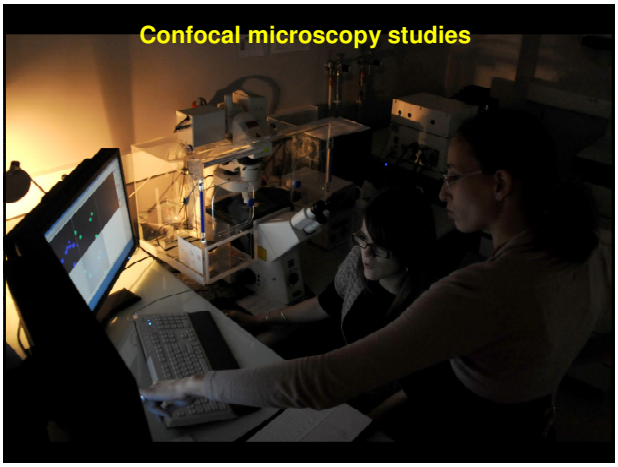


Modification of cell size and nuclei induced by CRAd and VPA



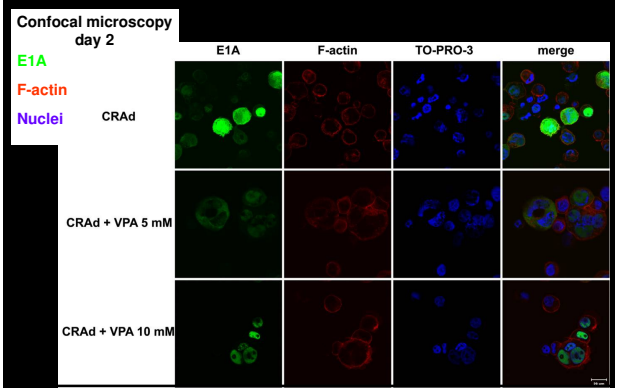
Wright-Giemsa staining day 3

Confocal microscopy studies



Confocal microscopy studies

Modification of cell size and nuclei induced by CRAd and VPA (HT29 cell line)



Modification of DNA repair by CRAd and VPA

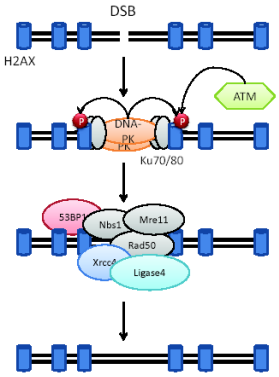
HDACi

- Inhibition of gene expression (Ku70, DNA-PK)

- Reduction of protein levels (Rad51, DNA-PK, CtIP, Exo1)

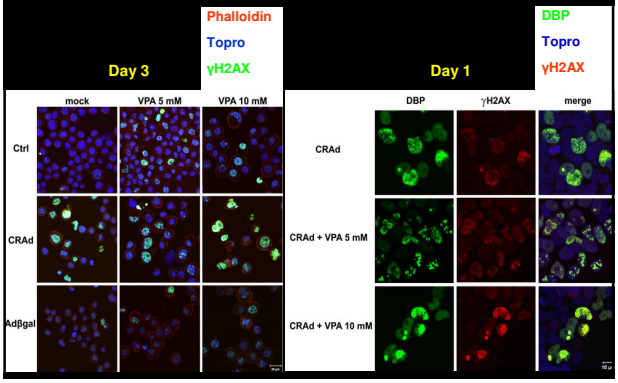
Adenovirus

- Inhibition of protein functions (MRE11, ligase IV and DNA-PK)



Quanz et al. PLOS One 2009

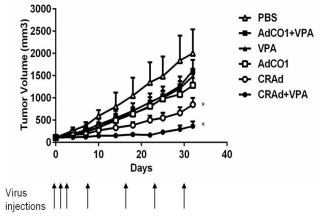
Co-treating HT29 cells with CRAd and VPA triggers strong DNA damage



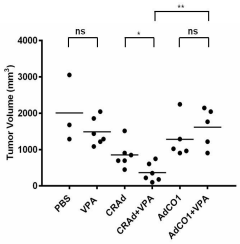
Reduction of tumor growth after cotreatment with CRAd and VPA

Virus subcutaneous (3 times and once/week)

VPA intraperitoneal (5/7 from day 4)



Tumor volume at sacrifice



Conclusion

✓ **Increased cell death** and **inhibition of proliferation** following cotreatment of colon carcinomas with CRAd+VPA

✓ The efficacy of the cotreatment is **not due to increased viral replication**

✓ Cotreatment with CRAd+VPA leads to the **appearance of a >4N population with increased cell size**. There are no subdiploid cells but some cells are **polyploid**

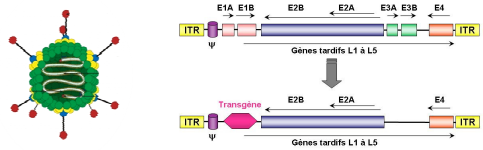
✓ Cotreatment with CRAd+VPA **strongly induced γH2AX**

✓ CRAd and VPA cotreatment translated into **a strong reduction of tumor growth**

Part 2

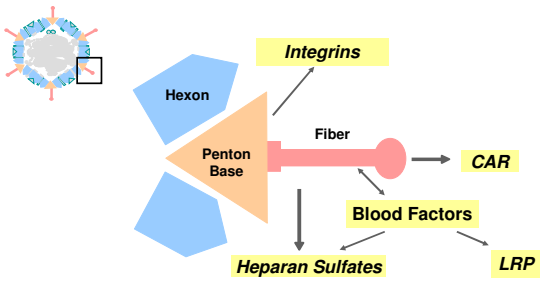
Monitoring gene transfer by adenoviral vectors using *in vivo* imaging

Adenoviral vectors (serotype 5, ΔE1ΔE3)



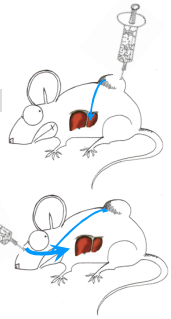
- Non-integrative
- Transduction of both post-mitotic and dividing cells
- Large cloning capacity (up to 7.5 Kb for ΔE1ΔE3)
- Production at high titer (10^{12} pfu/ml)
- Non-associated with serious human pathologies
- **Highly immunogenic**
- **Large tropism**

Ad5 tropism



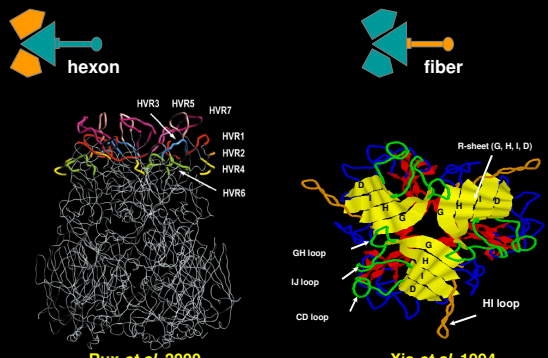
Limits to intratumoral gene transfer

- **High uptake by untargeted tissues (liver)**
  - upon systemic injection
  - after intratumoral delivery



- **Low or lack of expression of Ad receptors on tumor cells**

Structure of Ad major capsid proteins



Rux *et al.* 2000 Xia *et al.* 1994

Creation of new entry pathways

- Only **small peptides** could be inserted (structural constraints)
- Limited set of targeting peptides (RGD, K7, …)
- Need of new peptides

RGD — *Vigne et al.* Gene Ther. 1999; Majhen *et al.* J Gene Med. 2012

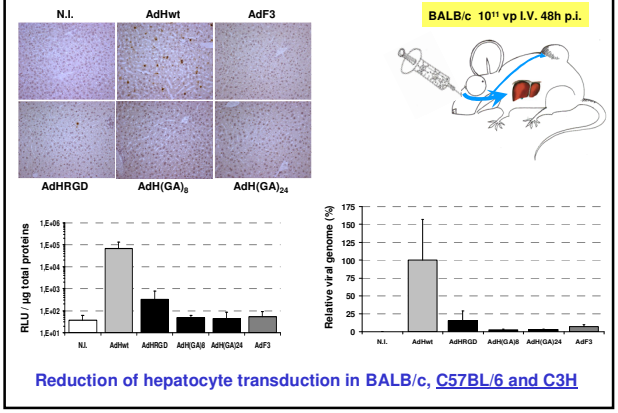
NGR — Jullienne *et al.* Gene Ther. 2009

1/ Controlling Ad uptake by untargeted tissues

- ***In vitro*** Ad infection is mainly dictated by CAR receptor
- ***In vivo***
  - Strong Ad liver tropism following intravenous injection
  - Unmodified by CAR or/and integrins mutations
  - Liver tropism is mainly due to interactions with blood factors

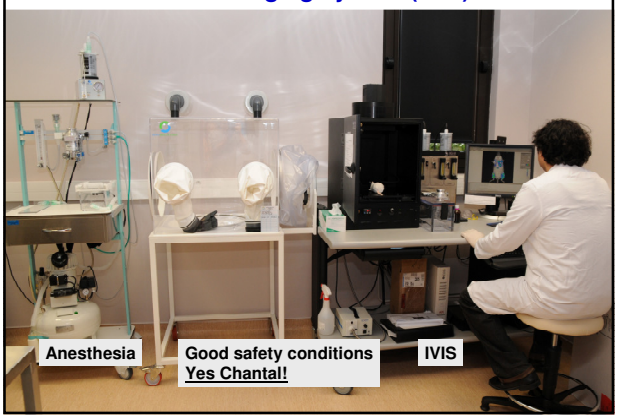
Descamps et al. Curr. Gene Ther. 2009

Gene transfer in liver following hexon modification



Reduction of hepatocyte transduction in BALB/c, C57BL/6 and C3H

In vivo imaging system (IVIS)



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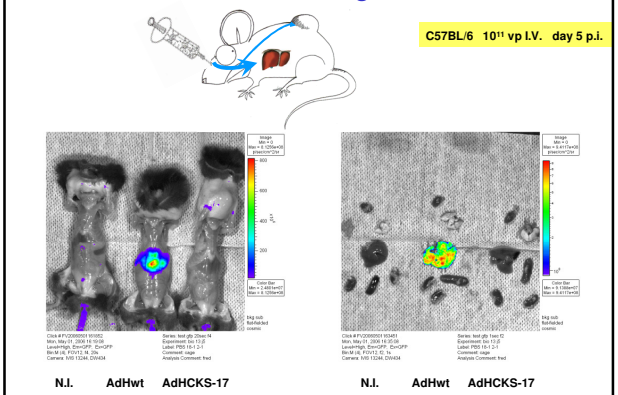
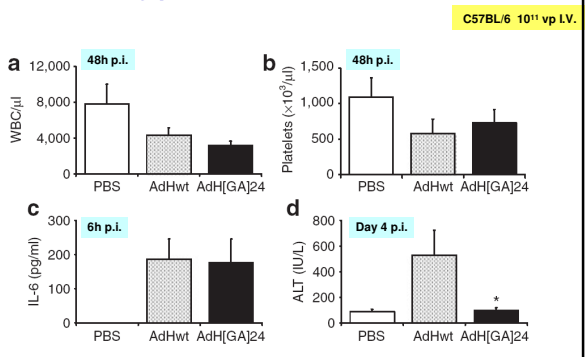


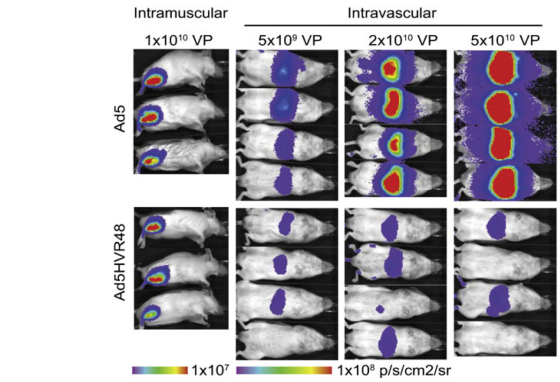
Toxicity profile of hexon-modified Ads



Reduction of liver toxicity (transaminases)

Gene transfer in liver following hexon modification

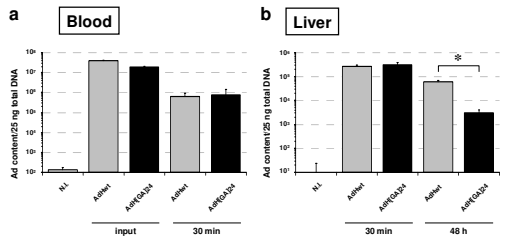
From Andy Baker's group



Clearance of hexon-modified Ads

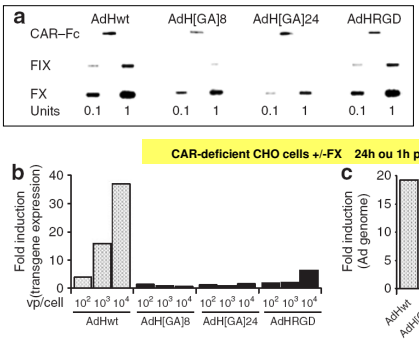
Real Time Q-PCR (viral genome)

C57BL/6 10^{11} vp I.V.



Similar uptake of viral DNA in liver but faster clearance

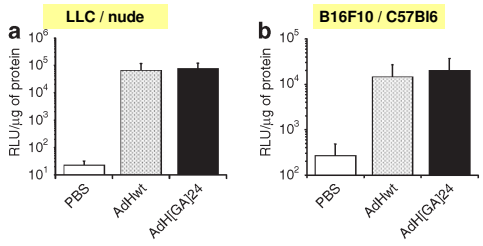
Hexon-modified Ad interaction with blood factors



Reduced binding to and use of blood factors by HVR5-modified Ad

Tumor transduction by HVR5-modified Ads

10^{11} vp I.T. 48h p.i.



Same level of gene transfer into tumors

Vigant et al. Mol. Ther. 2008

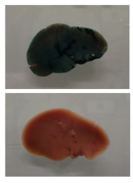
Gene transfer into adrenal glands

Collaboration with Georges Vassaux

- Defining the role of blood factors in adrenal gland transduction
- Defining the best way of administration to achieve efficient gene transfer

Role of blood factor in adrenal gland transduction

I.V. 10^{9} pfu, day 2 p.i.

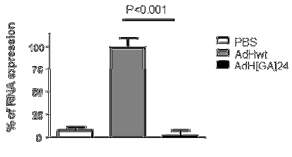


AdHwt

Liver

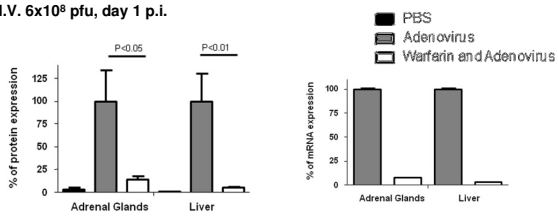
AdH[GA]24

Adrenal glands

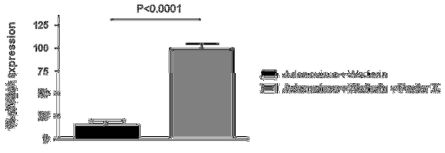


Role of blood factor in adrenal gland transduction

I.V. 6x10^{8} pfu, day 1 p.i.



I.V. 6x10^{8} pfu, day 2 p.i.



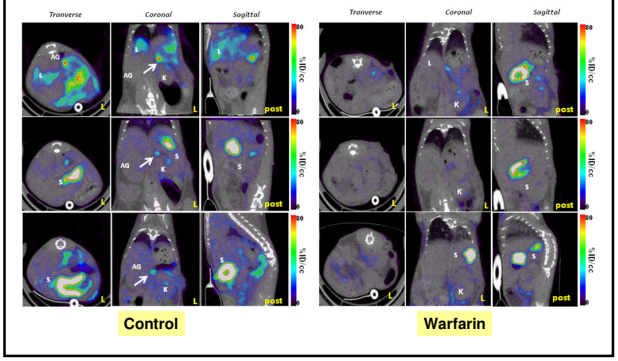
SPECT (single photon emission computed tomography) imaging of gene transfer

- AdCMVrNIS
- After 48h intraperitoneal administration of Technecium 99 (^{99}Tc 100MBq)
- Measurement of photon emission after 20min

SPECT imaging of gene transfer in the adrenal gland

AdCMV-rNIS 5x10^8 pfu, day 2 p.i.

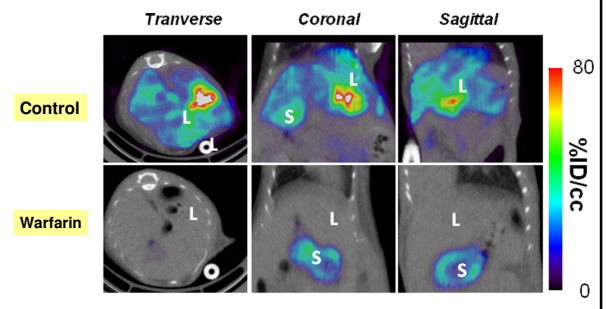
Intra-renal administration



SPECT Imaging of gene transfer in the liver

AdCMV-rNIS 5x108pfu, day 2 p.i.

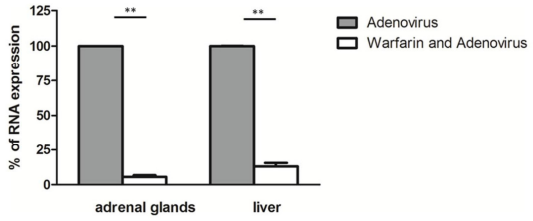
Intra-renal administration



Gene transfer in the liver and adrenal glands following intra-renal administration

AdCMV-rNIS 5x108pfu, day 2 p.i.
RT-QPCR

Intra-renal administration



Tran *et al.* PLOS One 2013

Conclusion

Use of different methodologies
Molecular biology + Biochemistry + immunostaining + imaging (fluorescence, luminescence and SPECT/CT)

Genetic or pharmacologic approach to inhibit blood factors

Liver
- Dramatically reduces gene transfer in liver and hepatotoxicity
- Early accumulation in liver remains unaffected, but clearance is faster
- Tumor gene transfer is unmodified

Adrenal glands
- Strong reduction of gene transfer following systemic or intra-renal administration

2/ Increasing gene transfer into tumors

- **Insertion of targeting peptides through genetic engineering**
  - **RGD peptide** *Majhen et al. J. Gene Med. 2012*
  - **NGR peptide** *Jullienne et al. Gene Ther. 2009*

- **Amplification of a replication-deficient recombinant adenovirus by an oncolytic adenovirus**

Acknowledgments

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Gaétan Cornilleau, Ingenior

Frédéric Vigant, PhD
Delphyne Descamps, PhD
Betsy Jullienne, PhD
Aleksandra Anchim, PhD
Najat Raddi, PhD

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